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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of : Aldo T. Iacono

Serial No. : 09/244,792 Art Unit: 1614

Filed : February 5, 1999 Examiner: Travers, R.

For : USE OF AEROSOLIZED CYCLOSPORINE FOR PREVENTION AND TREATMENT OF PULMONARY DISEASE

DECLARATION OF DR. ALDO T. IACONO
UNDER 37 C.F.R. 132

I hereby certify that this paper is being deposited with the United States Postal Service as First Class Mail in an envelope addressed to: Assistant Commissioner for Patents, Washington, D.C. 20231, on: April 22, 2003

Carmella L. Stephens — Attorney Name
Signature
41,328 — PTO Registration No.
06/13/03 — Date of Signature

Assistant Commissioner for Patents
Washington, D.C. 20231

I, ALDO T. IACONO, do declare:

1. I am inventor of the invention disclosed in the above-identified application. A copy of my Curriculum Vitae is attached herewith as Exhibit A.

2. The invention disclosed in the above identified application relates to methods and compositions for prevention of graft rejection in lung transplant recipients and for treatment of subjects with pulmonary disorders. Specifically, the invention relates to the

administration of *non-encapsulated* aerosolized cyclosporine, *i.e.*, non-liposomal cyclosporine, directly following transplantation for prevention of acute or chronic refractory rejection in lung transplant patients.

3. Disclosure of liposomal formulations of cyclosporine would fail to provide an expectation that non-encapsulated formulations of cyclosporine could be used successfully to prevent graft rejection or pulmonary inflammation. This is because one would recognize that liposomal formulations containing cyclosporine would have altered pharmacokinetic properties, such as biodistribution, clearance rates, and toxicity as compared to non-encapsulated formulations of cyclosporine since a lipid membrane surrounds the active drug product resulting in hydrophobic and hydrophilic aerosol droplet interactions.

4. A number of references could be viewed as teaching away from the use of non-encapsulated formulations of cyclosporine. For example, Knight (Exhibit B) states in column 2, lines 5-7 that "in laboratory animals the use of liposomes actually reduced toxic effects observed with the drug alone." Furthermore, as set forth in Gilbert (1996, J. of Aerosol Medicine 9:111-122: Exhibit C), "incorporation of potentially useful drugs into liposomes instead of using free drug has several advantages: solubility of lipophilic drugs allows for much greater concentrations of drug to be used; in many cases, incorporation decreases a drug's toxicity without affecting its inhibitory effects, and liposomal formulations may lead to better pharmacokinetics such that shorter and/or fewer treatments are necessary."

5. In addition, there are a number of potential drawbacks associated with the use of liposomal encapsulated formulations of cyclosporine that can be avoided by the use of the non-encapsulated cyclosporine compositions of the invention. For example, as set forth on pg.1573 of Harrington et al., (2002, Journal of Pharmacy and Pharmacology 54:1573; Exhibit D):

> "Formidable difficulties were presented by the need to produce stable drug-containing liposomes in a reliable, reproducible way. The entrapment conditions for any particular agent need to be optimized individually. Because liposomes can carry drugs in three compartments (water-soluble agents in the central aqueous core, lipid-soluble agents in the membrane, peptides and small proteins at the lipid-aqueous interface), a diverse range of optimal encapsulation conditions may exist for different agents. In addition, the release kinetics of the entrapped agents can vary, depending on the liposomal formulation, and this can effect the therapeutic efficacy. Therefore, development of agents for preclinical and clinical uses can be both laborious and expensive."

6. Furthermore, the results presented in Bridges et al, (2000, International Journal of Pharmaceutics 204:69-79: Exhibit E) demonstrate that selection of both nebulizer and liposome components of the nebulizer-liposome system are critical for drug delivery to the respiratory lung regions. Finally, as set forth in Desai et al. (2003, Pharmaceutical Research 20:442; Exhibit F), it was established that the encapsulation of polymyxin B sulfate, typically a systemic antibiotic, into liposomes reduced its antimicrobial activity indicating that encapsulation of a therapeutic agent into lipsomes can effect the efficacy of

the agent.

7. Experiments were conducted to demonstrate the effectiveness of non-encapsulated aerosolized cyclosporine as prophylaxis for lung transplant rejection. To measure the dose-response relationship, 15 subjects underwent a one time radionuclide study to measure the lung deposition of cyclosporine. Deposition in the transplant was correlated with physiologic indices of effectiveness as measured by the change in one-second forced expiratory volume (FEV1) over time. Deposited dose in the periphery of the transplanted lung(s) was compared to percentage change in FEV1 at post-operative days 200, 400 and 600. As indicated in Exhibit G, there was a significant correlation between improvement in FEV1 and dose at all time points.

8. Exhibit H shows the average percentage increase in pulmonary function for the group of single lung recipients who deposited $\geq$ 5 mg in the periphery of their transplanted lung(s) (the high dose group). Data is also presented for single lung recipients in the < 5 mg low dose group, and single lung recipients in the placebo. As demonstrated, subjects depositing 5 mg or more experienced an improvement in lung function, whereas placebo subjects, and subjects depositing less than 5 mg tended to demonstrate a decline.

9. Bronchial obliterans (OB) is the principle obstacle to long term survival after lung transplantation. Experiments were conducted to determine whether

aerosolized cyclosporine could confer a survival advantage in lung transplant recipients with OB. 39 lung transplant recipients with histologic OB received rescue therapy with aerosolized cyclosporine for refractory rejection. Survival was compared to two contemporaneous control groups with OB: 51 transplant recipients from the University of Pittsburgh and a multicenter group of 100 recipients from the US, Europe and Australia. The median survival after OB was 4.5 yrs with aerosolized cyclosporine versus 2.4 and 2.3 yrs in the Pittsburgh and multicenter controls. The data indicates that aerosol cyclosporine provides a survival advantage in lung transplant recipients with bronchiolitis obilerterans.

10. A set forth in Exhibit I, an increase in the survival rate of patients treated with aerosolized cyclosporine was observed as compared with placebo treated patients further demonstrating the successful use of non-encapsulated aerosolized cyclosporine for treatment of transplant recipients.

11. Despite the teaching away of the use of non-encapsulated cyclosporine as indicated above, the data presented herein demonstrates the successful use of non-encapsulated aerosolized cyclosporine as a prophylaxis for lung transplant rejection.

12. I hereby declare further that all statements made herein by my own knowledge are true and that all statements made on information and belief are believed to be true and further that I make these statements with the knowledge that willful false statements and the like are punishable by fine or imprisonment, or both, under ' 1001 of Title 18 of the

united States Code and that such willful false statements may jeopardize the validity of the application of any patent issuing therein.

7/29/03
Dated:

Dr. Iacono